1973

Pathogenicity, Synergism, and Control of Xanthomonas Translucens and Septoria Avenae f. sp. Triticea on Spring Wheat in South Dakota

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PATHOGENICITY, SYNERGISM, AND CONTROL OF *XANTHOMONAS TRANSLUCENS* AND *SEPTORIA AVENAE* F. SP. *TRITICEA* ON SPRING WHEAT IN SOUTH DAKOTA

BY

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A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in
Agronomy, South Dakota
State University

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser Date

Head, Plant Science Department Date

ACKNOWLEDGEMENT

I wish to thank Dr. G. W. Buchenau for suggesting the thesis problem and for his constructive criticism during the course of this study. I am also indebted to Dr. C. J. Mankin and Dr. J. D. Otta for their aid in identification and culturing of the fungus and bacterium used in this thesis.

TABLE OF CONTENTS

INDEX OF TABLES

INDEX OF FIGURES

INTRODUCTION

During the 1969 growing season an unusual leaf disease developed extensively on spring and winter wheats (Triticum aestivum L.) in experimental plots at Brookings. This disease was characterized by symptoms somewhat similar to those of three common but relatively minor wheat diseases; Septoria leaf blotch, bacterial blight, and Helminthosporium leaf spot. Though similar to each, symptoms were not truly typical of any one disease. Symptoms similar to those observed at Brookings had been observed elsewhere in South Dakota prior to 1969.

A fungus tentatively identified as Septoria avenae Frank f. sp. triticea Johnson and a bacterium designated as Xanthomonas translucens Dowson were observed in, or isolated from, many such lesions. The abundant atypical symptoms and the presence of both pathogens suggested the possibility of a synergistic relationship between the two organisms.

Since relatively little was known about the nature of the Septoria species or about its relationship to X. translucens, this study was conducted to answer several questions relating to the problem:

1. What is the proper identity of the Septoria species?
2. Is there a synergistic relationship between this Septoria and X. translucens?
3. Do spring wheat cultivars currently grown in South Dakota have resistance to this disease complex?
4. What available pesticides, if any, will control this disease complex?

LITERATURE REVIEW

The bacterium Xanthomonas translucens is classified in the order Pseudomonadales and in the family Pseudomonadaceae which includes the genera Pseudomonas, Acetobacter, and Xanthomonas. General characteristics of the family are as follows: short to medium-length gram-negative rods which do not produce spores, aerobic, heterotrophic, motile with polar flagella, and occur in nature in soil and water. Specific characteristics of the genus Xanthomonas include: monotrichous cells which produce yellow-non-water-soluble pigment on agar, readily digest proteins, produce hydrogen sulfide, produce acid from monosaccharides and disaccharides, increase the pH of milk, and are mostly plant pathogens causing necrosis (23, 7).

Identification of plant pathogenic bacteria by routine bacteriological methods is laborious, time consuming, and sometimes unreliable. For a number of years researchers have been searching for alternative methods of identification in order to investigate more effectively the salient features of host-parasite relationships such as epidemiology.

Wernham (38) attempted to identify various species of Xanthomonas by cross inoculation of 17 Xanthomonas isolates on 16 taxonomically distinct hosts. His tests indicated that Xanthomonas isolates could be partially identified by host range and reaction. His results also showed that methods of inoculation and pre-and post-inoculation environment determined reaction type and whether a given plant was susceptible or resistant.

The incitant of bacterial blight on a number of cereals and grasses was originally named Phytomonas translucens (5). Hagborg (12) revised this classification and designated the organism Xanthomonas translucens. Pathogenicity represented a basis for additional subdivision within the species and further study resulted in the elucidation of 4 form-species: X. translucens f. sp. hordei, f. sp. secalis, f. sp. hordei-avenae, and f. sp. cerealis (5, 6).

Fang (11) further refined Hagborg's system and proposed the following reorganization of the form-species based on his pathogenicity studies on cereals and grasses:

f. sp. hordei attacks barley

f. sp. undulosa attacks wheat

f. sp. secalis attacks rye

f. sp. cerealis attacks smooth brome and quack grass

f. sp. phleipratensis attacks timothy

Bacterial blight occurs on leaves and possibly on floral bracts and culms of wheat, rye, and some grasses. The organism overwinters in crop residue and on the seed and can survive unfavorable conditions in the form of dehydrated exudate for long periods of time (10). Epidemiology studies by Wallin (34) showed that the organism overwintered in the tissue of the perennial grasses timothy (Phleum pratense), and smooth brome grass (Bromus inermis). Wallin concluded that Bromus inermis was the most important overwintering host of the form-species that attacks wheat. Boosalis found that X. translucens also overwintered in the soil and on barley and wheat seed (2). Later Boosalis

found that X. translucens isolates from B. tectorum (Downy chess) and B. commutatus (Hairy chess) were weakly virulent on wheat, implicating these two grasses as overwintering hosts for the form-species that attacks wheat. However, he concluded that the major sources of overwintered (primary) inoculum were infected plants of barley, wheat, rye, smooth brome grass, and quack grass (Agropyron repens) (4).

Primary infections occur during the seedling stage in the early spring after several days of rainy, damp weather. Secondary infections occur on young tissue throughout the growing season whenever high moisture conditions prevail. The bacterial exudate is splashed by rain, transmitted by contact, and carried by insects to host tissue (2).

Bacteria enter host tissue through natural openings and wounds. Once the bacteria have gained entrance into host tissue, symptoms begin to appear in 7-10 days. Young lesions are water-soaked in appearance and upon maturation light yellow droplets or scales of exudate appear on the surface of lesions. Older leaf lesions are light brown, translucent in the centers, irregularly linear, and frequently coalesce to form blotches (10).

Bacterial blight of wheat is of minor importance in most years. However, sporadic outbreaks occur when environmental conditions and sufficient overwintered inoculum favor seedling infection in the spring. Secondary infection occurs as long as environmental conditions remain favorable for disease spread and growth (10). Boosalis demonstrated that prevalence and severity of X. translucens on wheat were dependent on environment and cultivar attacked (2).

Recommended control of bacterial blight consists of crop rotation, seed treatment with mercury compounds, and resistant cultivars (10). Hagborg (13) showed resistance of Thatcher wheat seedlings to infection by X. translucens was increased by the use of Streptomycin. Streptomycin has controlled bacterial diseases caused by related Xanthomonads (8, 22).

A number of techniques have been used to artificially inoculate plants with X. translucens including inoculation through wounds, brushing or atomizing bacterial suspensions onto the foliage, and vacuum infiltrating leaf tissue with bacteria (2, 11, 38). Wound and atomization techniques have resulted in satisfactory infection levels, but symptom expression has varied. Boosalis (3) vacuum infiltrated suspensions of X. translucens into seedlings and found this method to be a very efficient technique for inoculating large numbers of plants uniformly and screening cultivars for resistance. He also used the vacuum infiltration principle to detect the presence of small numbers of naturally surviving bacteria in soil and debris.

Leaf blotch of wheat caused by Septoria avenae f. sp. triticea is a disease of minor importance in most years. When environmental conditions are suitable, the pathogen can cause severe infections on wheat. Weber described Septoria diseases on wheat, rye, barley, and grasses and conducted fundamental studies on the host-parasite relationships (35, 36, 37).

Taxonomically, the genus Septoria belongs in the form-class Deuteromycetes (Fungi Imperfecti) and in the form-order Sphaeropsidales.

These fungi have septate mycelium and reproduce by means of conidia. Most of the members of the Deuteromycetes have no sexual stages and their conidial stages closely resemble the conidial stages of Ascomycetes. Therefore, the Deuteromycetes are considered as conidial stages of Ascomycetes whose sexual stages have been lost through evolution or have not been discovered (1).

The sexual stage of the organism that causes the leaf blotch disease on wheat has been found in nature, but only rarely. The correct name and classification of the Septoria leaf blotch organism when based on the sexual stage is Leptosphaeria avenaria (20). Because the sexual stage L. avenaria is rarely found in nature, the name of the imperfect stage S. avenae f. sp. triticea is used herein. S. avenae f. sp. triticea accurately defines the stage of the pathogen that was observed and manipulated during the course of my studies and such usage has precedent in the literature (20).

The most common Septoria diseases of wheat are leaf spot and glume blotch, caused by S. tritici and S. nodorum respectively. Much of the literature relates to these species and also to S. avenae on oats and relatively little is known about S. avenae f. sp. triticea.

Johnson (20) described a form of Leptosphaeria on wheat in Canada which he designated S. avenae f. sp. triticea which differed from S. nodorum in that it had longer pycnidiospores. It differed from S. avenae in host range, length of incubation period and cultural characters, and its spores were slightly longer and thinner. Each pathogen produced symptoms which were distinct from one another.

Hosford et al. (18) reported the occurrence of S. avenae f. sp. triticea in North Dakota. Isolates usually formed the pycnidial stage in the lesions and the perfect stage was occasionally found.

Richardson and Noble (25) have illustrated and summarized the characteristics of Septoria species on cereal hosts in Scotland.

Leaf blotch occurs widely on wheat and on some grasses. The pathogen overwinters as spores or mycelium in living or dead host tissue. Spores inside pycnidia and mycelium within the host tissue persist for long periods of time in crop residue. Leaf infections occur in the fall or spring, especially during cool, wet weather. Pycnidia absorb moisture, swell and eventually expose their spores in a gelatinous matrix to the environment. The spores are then transmitted by rain, insects, and mechanical contact to susceptible host plants (10). After spores have been deposited on a suitable infection court, they germinate and germ tubes penetrate into the plant tissue; these events require a period of free moisture of 36-48 hours (17).

Once inoculation has taken place and infection has been established, leaf blotch symptoms first appear as ovate, straw or buff colored lesions. Lesions spread and coalesce rapidly to form light brown, irregular blotches with a speckled appearance as the gray-black pycnidia develop in the central portion of the lesion (10).

Control measures include crop rotation, sanitation, and plowing under volunteer wheat plants in the fall. There are few known resistant wheat cultivars in the United States (10).

Early in the study I encountered problems in producing inoculum and in obtaining uniform symptom development on inoculated plants. It was then necessary to gain more information about inoculum production, inoculation procedures and symptom development. Examination of the literature revealed little information specifically about S. avenae f. sp. triticea; therefore, it was essential to study related Septoria species to gain insight into these problems.

Most Septoria species adapt easily to growth on many types of artificial media. In many cases abundant mycelial growth was obtained with slight or no spore production. In my experience with S. avenae f. sp. triticea, the isolates sporulated abundantly in the first few transfers but became nonsporulating mycelial colonies on subsequent transfers. Other workers have expended considerable effort to induce sporulation and to maintain pure stable cultures of Septoria species (9, 14, 16, 24).

Scharen and Krupinsky (28) found that S. nodorum had cultural variability into the ninth single-spore generation. Variable characters were mycelial color, growth, number and formation of pycnidia, spore morphology and pathogenicity. Cultures that originally produced many pycnidia and spores gave rise to mycelial types that produced no spores after three transfers.

Hooker (16) found that the variable fungus, S. avenae, produced many cultural types on artificial media. Cultures isolated from naturally infected hosts were primarily mycelial types on potato-dextrose agar. Single spore transfers produced more pycnidial growth

in less time than mass spore transfers. Successive single spore transfers of selected segregates resulted in stable sporulating cultures.

Mycelial cultures of S. nodorum have been induced to sporulate when grown under artificial light (9, 24). Richards (24) found light intensity of 100 ft-c and above from a 15 watt daylight type fluorescent lamp increased sporulation in proportion to light intensity when applied to cultures for 5 days at 20 C.

Calpouzos and Lapis (9) demonstrated that light was needed to initiate pycnidial formation and also at a later date to initiate spore production. Light requirement for initiating both processes varied for different isolates. Wave lengths shorter than 350 nm in the near ultraviolet induced pycnidial formation.

Other techniques to obtain sporulation have been successful. Nutritional stress from heavy seeding of growth media resulted in improved sporulation of S. nodorum. Organic nitrogen source also was involved in the sporulation process (24). Hilu and Bever (15) tested a number of growth media and found that S. tritici sporulated most abundantly on Elliot's V-8 agar.

Several inoculation methods have been used with Septoria species (18, 21, 26, 35, 36, 37). Hooker (17) working with Septoria diseases of oats, tested a number of techniques and preferred to blend sporulating cultures with water and spray this suspension onto the foliage, followed by incubation in a dew chamber for 36 hours. Fresh cultures produced more infection than cultures more than 16 days old. He also

demonstrated that sunlight during the dew and inoculation periods resulted in decreased infection.

In field tests Hilu and Bever (15) obtained differential cultivar reactions by water-soaking wheat leaves with a dilute spore suspension of S. tritici. An inoculum concentration of 10-15 spores per 430 X microscope field gave the best differentiation of resistant cultivars; higher concentrations resulted in poorer differentiation.

Scharen (27) used hypodermic inoculations of S. nodorum on plants that ranged in size from seedling to shooting and found that infections were confined to leaves and those portions of culm tissue that were in direct contact with injected spores. He later studied the effect of humidity treatments on plants following inoculation with S. nodorum and demonstrated that a "dew" period of at least 48 hours was needed for germination, penetration and successful establishment of infection hyphae (29).

MATERIALS AND METHODS

Production of Inoculum- Mycelial cultures of S. avenae f. sp. triticea were obtained from dried leaf specimens when leaf pieces were surface disinfested in 70% alcohol for 5 minutes and placed on potato dextrose agar (PDA) slants (32). Preliminary attempts to induce sporulation eventually led to the following procedure for maintaining sporulating cultures of the organism. Hyphal segments from mycelial cultures on PDA slants were grown on V-8 Juice agar (32) under continuous 100-150 ft-c fluorescent light until mature pycnidia developed. These pycnidia were crushed in a drop of sterile water and the spores streaked on PDA plates; 24 hrs later one germinated spore was placed on a V-8 agar slant. After a succession of these single spore transfers, the time period required for pycnidia maturation was reduced from the initial 25-30 days to 13 days (Figure 1).

For all tests Septoria was grown on V-8 juice agar slants and maintained as pure sporulating cultures by single spore transfers. These single spore cultures were allowed to grow under existing laboratory light conditions for 7 days after which they were put under continuous artificial light for 6 days.

X. translucens was isolated from lesions on brome grass and quack grass by streak plate techniques on PDA and subsequently transferred to King's Medium B agar (32) for routine maintenance of stock cultures and for inoculum production. Growth characteristics and

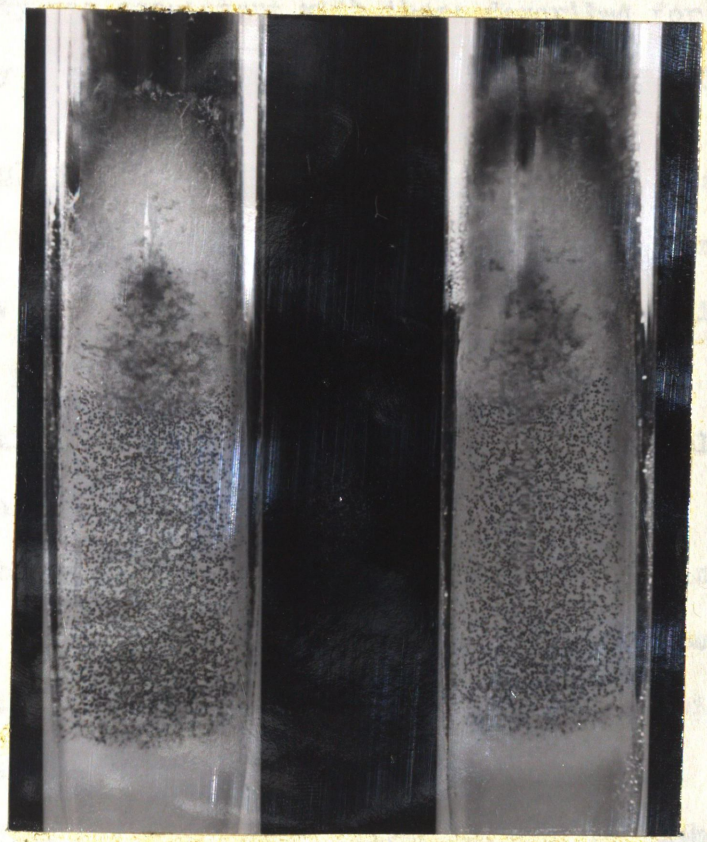
Figure 1. V-8 agar culture of Septoria avenae f. sp. triticea sh
mature pycnidia on slant surface.

A - Close up of pycnidia (approximately 2X)

B - Close up of pycnidia (approximately 4X)

Figure 1-

A



B

pathogenicity was consistent with those described for X. translucens. The identity of this organism was verified by Dr. J. Otta, SDSU.[1]

Inoculation Methods- In preliminary tests to establish suitable inoculation techniques, I evaluated several methods and found that infection by both pathogens was greatest when vacuum infiltration was used with a post-inoculation dew period of 24 hours. More than 24 hours in a mist chamber did not result in higher levels of disease in greenhouse experiments.

Air-blasting with inoculum suspension containing celite was second only to the vacuum infiltration process in amount of infection. The air-blast technique was chosen for field inoculations because it provided the advantages of simplicity and uniformity.

Inoculum for field experiments was applied with a portable gasoline operated compressor at a pressure of 60-80 psi. Celite (1%) was used as an abrasive in the inoculum suspension and in the water control.

Septoria inoculum for field and greenhouse tests was adjusted to a concentration of $2\text{-}3 \times 10^5$ spores/ml and Xanthomonas to an OD reading of 0.35-0.40. The latter corresponds to a concentration of 1×10^6 cells/ml as determined by dilution plating.

Greenhouse inoculations were initiated by planting 15 wheat seed in paper towel "boats" filled with vermiculite. After emergence the stand was thinned to 10 plants per boat. Boats containing 2-3 week old

[1]Dr. J. Otta, Plant Pathologist, South Dakota State University

seedlings were placed in a desicator containing a water suspension of the pathogens and arranged so that the leaves were submerged. Concentrations of inoculum used were the same as those previously described for field use. The dessicator was evacuated for 15 minutes with a water tap aspirator. The vacuum was slowly released and the inoculum soaked seedlings were placed in a mist chamber for 24 hours. The boats were then placed in sand benches and the plants and greenhouse bench were covered with plastic for 2-3 days. Humidity remained high and temperature fairly constant, around 21-26 C. Symptoms developed in 6-7 days for both diseases. Visual estimates of the percentage of leaf area diseased were conducted according to standards developed by James (19). These standards, incidentally, were contributed by Dr. James prior to publication.

RESULTS

Identity of Septoria Species- The identity of the Septoria species involved was considered tentative at the outset. Consequently, further study was required to affirm the identity of the organism. Leaves infected with Septoria leaf blotch collected by Dr. G. Buchenau in the summer of 1969 contained pycnidia that ranged in size from 133-160 μ dia and pycnidiospores that ranged from 14.6-20.0x4.0-5.3 μ 3 septate rarely 1, 2, or 4. The size, shape and color of pycnidia and pycnidiospores partially agreed with Johnson's (1948) description of S. avenae f. sp. triticea, i.e. pycnidia 90-140 μ dia and pycnidiospores 18-53x2.3-4.2 μ, 3 septate rarely 4 (20).

Several Septoria species and related fungi occur in nature which are morphologically similar. Some diagnostic characteristics of these organisms are presented in Table 1. S. tritici produces both macrospores (35-98x1.4-2.8 μ) and microspores (5.9x0.3-1.0 μ); the former are longer and narrower than S. avenae spores. S. avenae f. sp. triticea spores are generally shorter and more slender than S. avenae spores. S. nodorum spores are somewhat shorter than S. avenae spores but wider than S. tritici spores. Stagnospora avenaria spores are wider than any of the Septoria species in Table 1 and longer than all but S. tritici. Pycnidia of S. avenae, tritici, and avenae f. sp. triticea are about the same size, with S. nodorum being the largest and Stagnospora avenaria the most variable of the group. Pycnidial color for all species varies from brown to black and pycnidiospores generally have three or four septa.

Table 1. Diagnostic characteristics of several Septoria species*

Organism	Pycnidial size (dia. μ)	Pycnidial color	Pycnidio-spore size (μ)	Pycnidio-spore septation	Pycnidio-spore structure
S. avenae	90-150	brown to black	25-45 x 3-4	3	bacillar, obtuse to round ends
S. tritici	100-150	golden brown	35-98 x 1.4x2.8	4	macrospores
S. avenae f. sp. triticea	90-140	golden brown to black	26-42 x 2.8-3.5	3 rarely 4	cylindrical and straight, obtuse or rounded ends
S. nodorum	160-210	light brown to black	15-32 x 2-4	0 to 3	short cylindrical, wedge shaped, obtuse
Stagnospora avenaria	50-160	golden brown	25-60 x 2.5-5.0	Usually 3 (can be 1-4)	cylindrical to slight subfusiform, rounded ends

*Adopted from Sprague (31)

Table 2 depicts the size of pycnidia and pycnidiospores found in other workers' studies on S. avenae f. sp. triticea. The average range of pycnidiospore dimensions in the four studies was 22.3-36.5x3.0-4.7 μ. Spores usually had 3 or 4 septa. Pycnidial dimensions were fairly constant within the interval 90-178 μ dia.

All characteristics and dimensions contained in Tables 1 and 2 indicate that the test organism used in this thesis was S. avenae f. sp. triticea.

Table 2. Descriptions of pycnidia and pycnidiospores of S. avenae f. sp. triticea

Source	Dimensions		Septation
	Pycnidial dia (μ)	Pycnidiospore (μ)	
1. Johnson (20)	90-140	18-53 x 2.3-4.2	3, rarely 4
2. Richardson (25)	---	30-50 x 3-4	4
3. Hosford (18)	93-178	19-48.5 x 2-5	3 rarely 1, 2, or 4
4. This study	133-160	14.6-20 x 4.0-5.3	3 rarely 1, 2, or 4

Inoculation Studies- Greenhouse inoculation tests were conducted to determine if inoculation by vacuum infiltration of seedling leaves (cultivar "Chris") with a mixture of X. translucens and S. avenae f. sp. triticea resulted in symptoms similar to those previously observed in the field. This was not the case, as symptoms from the inoculation combinations were characterized as rather typical mixed infections of bacterial blight and leaf blotch without any apparent synergistic effect on lesion type (Figure 2).

Symptoms that developed from inoculations with Xanthomonas started as separate water-soaked linear lesions, which subsequently coalesced to cover the outer 1/3 of the leaf blade from the tip back (Figure 2B). Septoria symptoms appeared as small ovate lesions, the central portion being light brown in color and containing black pycnidia. The surrounding tissue was light green-yellow in color (Figure 2C). Water inoculated control plants produced no visible symptoms (Figure 2A).

Disease severity, however, was affected by the composition of the inoculum. In experiment 1 bacterial blight development was more

Figure 2. Leaf lesions caused by Xanthomonas translucens and Septo
avenae f. sp. triticea infection.

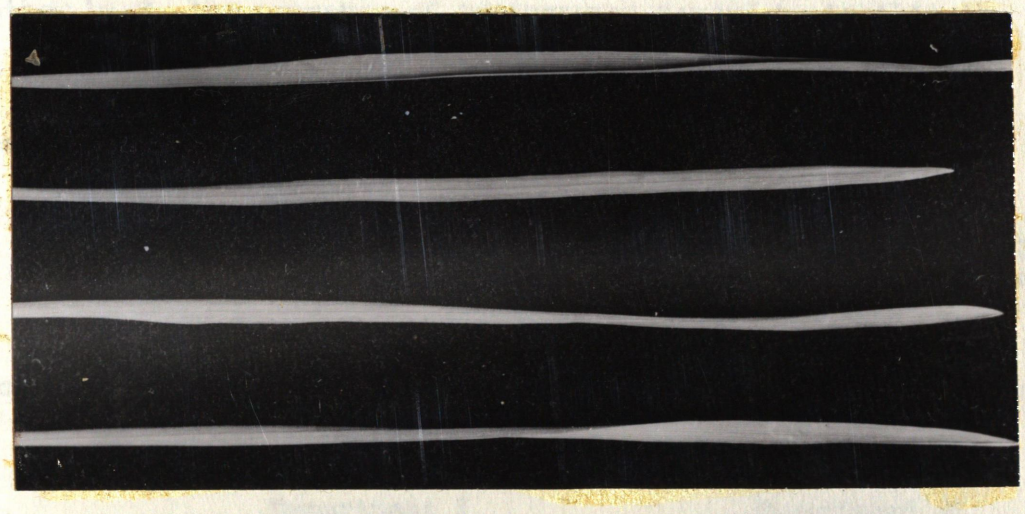
A - Control leaves.

B - Xanthomonas translucens infected leaves.

C - Septoria avenae f. sp. triticea infected leaves.

Figure 2-

A



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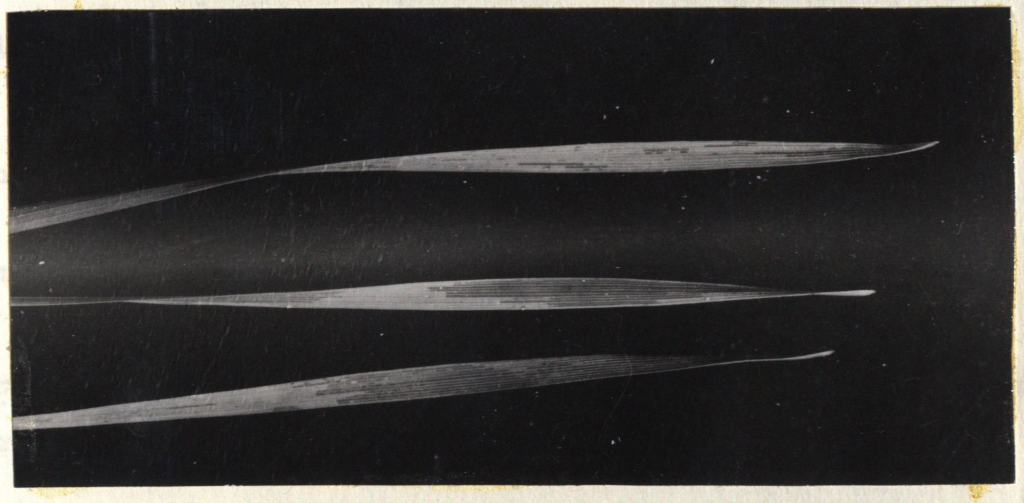
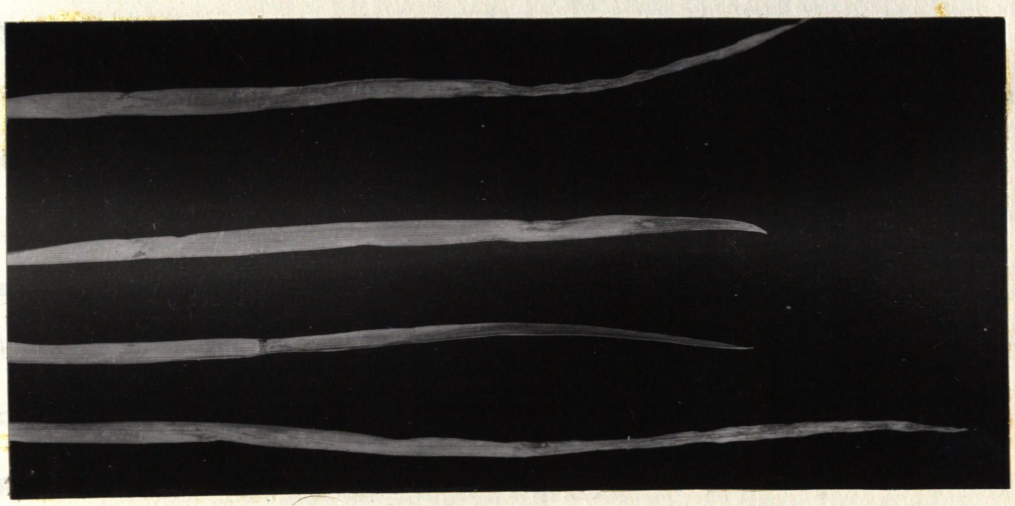


C



extensive when Septoria spores were present in the inoculum (Table 3). On the other hand, the presence of Xanthomonas tended to inhibit the severity of leaf blotch symptoms. Similar results were obtained in experiment 2 and both responses were significant at the 5% level.

Table 3. Effect of inoculum combinations on leaf infection by Septoria avenae f. sp. triticea and Xanthomonas translucens on Chris wheat under greenhouse conditions.

	Disease Rating (%Leaf Blade Infected)*			
	Experiment 1		Experiment 2	
Inoculum Composition	Leaf Blotch	Bacterial Blight	Leaf Blotch	Bacterial Blight
Septoria alone	2.10a	---	19.9a	---
Xanthomonas alone	---	0.63b	---	3.90b
Septoria & Xanthomonas	1.60a	5.70a	3.60b	4.50a
Control	.00b	0.03b	0.38c	0.10c

*Means of 60 leaves covered by the same letter are not significantly different at the .05 level using Duncan's Multiple Range test.

Further studies were conducted in an attempt to elucidate the interrelationships of these two pathogens under field conditions. Chris spring wheat was grown in four row plots, 12 ft long. Treatments consisted of inoculations with Xanthomonas alone, Septoria alone, a combination of the two, and a water control arranged in a randomized complete block design with 5 replications. The foliage of the inner two rows of each plot was air-blasted with the indicated inoculum containing 1% celite as an abrasive on each of four inoculation dates:

29 May, 9, 17 and 29 June, 1970. Ten-culm samples were taken from each plot prior to each inoculation and again on 10 July, 1970 and each of the top four leaves on each culm was rated visually for disease development.

Differences in Septoria leaf blotch development were not detected until 29 June when Septoria inoculated leaves had developed more abundant symptoms than control leaves (Table 4). Leaf blotch development on leaves 2 and 3 at all dates was generally slightly higher from the inoculum mixture than from Septoria inoculum alone.

Differences in bacterial blight development were not detected until 29 June when all Xanthomonas inoculated leaves had more infection than control leaves. By 10 July leaves 2 and 3 had consistently (although not significantly) more bacterial blight symptoms from mixed inoculum than from Xanthomonas inoculum alone. These results are consistent with the greenhouse results. Severity of both diseases increased with time and symptoms were more abundant on lower leaves.

The effect of inoculum composition on the rate of leaf blotch and bacterial blight development was evaluated by computing rate of increase (r) as described by Van Der Plank (33). Rates of increase (r values) for leaf blotch and bacterial blight (Table 5) indicate that an inoculum combination of Septoria and Xanthomonas resulted in a more rapid increase of bacterial blight on the second leaves than did inoculum composed of Xanthomonas alone. This effect was not evident on other leaves. Leaf blotch symptoms developing from the inoculum

Table 4. Effect of inoculum combinations on leaf infection by Septoria avenae f. sp. triticea and Xanthomonas translucens on Chris wheat under field conditions.

Inoculum Composition	Disease Rating (% Leaf Blade Infected)*							
	Leaf Blotch				Bacterial Blight			
	6/9	6/17	6/29	7/10	6/9	6/17	6/29	7/10
	Flag Leaf							
Septoria alone	.00a	.00a	.01a	1.8a	---	---	---	---
Xanthomonas alone	---	---	---	---	.12a	.00a	1.1a	7.7a
Septoria & Xanthomonas	.00a	.00a	.04a	1.7a	.14a	.00a	1.2a	5.3a
Control	.00a	.00a	.01a	1.0b	.15a	.00a	.29a	4.7a
	Second Leaf							
Septoria alone	.01a	.01a	.50a	5.0a	---	---	---	---
Xanthomonas alone	---	---	---	---	.11a	.02a	2.2a	30.3a
Septoria & Xanthomonas	.03a	0a	.69a	5.9a	.12a	.00a	2.4a	47.6a
Control	.01a	0a	.12b	3.2b	.27a	.01a	.48b	38.9a
	Third Leaf							
Septoria alone	.15a	.43a	1.8a	4.6ab	---	---	---	---
Xanthomonas alone	---	---	---	---	.40a	1.4a	11.2a	47.6a
Septoria & Xanthomonas	.71a	.34a	1.6a	5.5a	.14b	.76a	10.9a	60.1a
Control	.18a	.13a	1.3a	2.7b	.17b	1.2a	5.0b	50.8a

* Means of 50 leaves covered by same letter are not significantly different at the .05 level using Duncan's Multiple Range test. Comparisons valid on a single leaf at one sampling date.

mixture developed at a slower rate than from the Septoria inoculum alone for all leaves. These trends are consistent with greenhouse results.

Table 5. Effect of inoculum composition on the rate of increase of leaf blotch and bacterial blight on leaves of Chris wheat.

Inoculum Composition	Rate of Increase (r)*	
	Leaf Blotch	Bacterial Blight
	Flag Leaf	
Septoria alone	.43	---
Xanthomonas alone	---	.13
Septoria and Xanthomonas	.31	.12
Control	.38	.10
	Second Leaf	
Septoria alone	.20	---
Xanthomonas alone	---	.21
Septoria and Xanthomonas	.17	.30
Control	.19	.21
	Third Leaf	
Septoria alone	.12	---
Xanthomonas alone	---	.25
Septoria and Xanthomonas	.07	.22
Control	.10	.21

* $$r = \frac{\log_e \frac{x_2}{1-x_2} - \log_e \frac{x_1}{1-x_1}}{t_2 - t_1}$$ where x_1 = disease rating at data 1 (t_1) and x_2 = disease rating at date 2 (t_2)

Resistance of Cultivars- A study was undertaken to determine if resistance to S. avenae f. sp. triticea and X. translucens infection is present in commercial spring wheat cultivars adapted to South Dakota. Greenhouse inoculations were conducted by vacuum infiltrating seedlings with a water suspension of a mixture of Xanthomonas and Septoria propagules. Disease readings were made 6-10 days after inoculation.

The cultivars most resistant to bacterial blight were Manitou, Chris, Hercules, Sheridan and Polk (Table 6). The cultivars most resistant to leaf blotch were Manitou, Hercules, Era, Sheridan, Leeds, Polk, and Chris. The cultivars most susceptible to both diseases were Crim, RR-68, Waldron, Fortuna, and Justin. Septoria infection was generally higher than Xanthomonas infection on the same leaf. Cultivars severely infected by one disease were also severely infected by the other.

Table 6. Reaction of spring wheat cultivars to a mixture of Septoria avenae f. sp. triticea and Xanthomonas translucens under greenhouse conditions.

	Disease Rating (% Leaf Blade Infected)*	
Cultivars	Bacterial Blight	Leaf Blotch
Crim	10.30a	12.40a
RR-68	9.05ab	7.60ab
Waldron	4.90ab	5.30abc
Fortuna	3.30bcd	5.10abc
Justin	2.53cde	3.45bc
Leeds	1.25cde	1.60c
Thatcher	1.25cde	2.81bc
Era	1.21cde	1.35c
Neepawa	1.13cde	1.65c
Selkirk	.96cde	2.33bc
Polk	.85cde	1.80bc
Sheridan	.36de	1.40c
Hercules	.32de	.75c
Chris	.18de	1.80bc
Manitou	.08e	.71c

* Readings for both diseases taken on same leaves. Means of 60 leaves covered by same letter are not significantly different at the .05 level using Duncan's Multiple Range test.

In addition to greenhouse experiments, field tests were conducted to determine cultivar reactions of spring wheats to an inoculum mixture of Xanthomonas and Septoria. Fifteen common spring wheat cultivars were planted in single row plots 12 ft long in a randomized complete block design with 3 replications. On 29 May, 1971, foci of disease were established on the first three feet of foliage in each plot and permitted to spread naturally from these foci. The foci were reinoculated on three occasions: 9, 17 and 29 June.

Disease readings were made on four different occasions: 11, 19, 32, and 45 days after the first inoculation (Table 7). No disease had developed by day 11 and readings from the last sampling date were confounded by severe rust development on some cultivars. On day 19 slight disease had developed on the flag leaves of Fortuna, Crim, and Leeds. The data indicated that cultivars displaying the most resistance to bacterial blight were generally resistant to leaf blotch as well, and conversely, cultivars susceptible to bacterial blight were generally susceptible to leaf blotch. Over all, the cultivars most resistant to leaf blotch and bacterial blight were Leeds, Hercules, Era, RR-68 and Chris; while the most susceptible cultivars were Thatcher, Fortuna, Crim, and Neepawa. Xanthomonas infection was generally higher than Septoria infection on the same leaf.

The resistance of cultivars to leaf blotch and bacterial blight was evaluated further by comparing the rate of disease increase (r) that occurred on them (33).

Table 7. Reaction of spring wheat cultivars to an inoculum mixture of Septoria avenae f. sp. triticea and Xanthomonas translucens under field conditions.

Cultivars	Disease Rating (% Lead Blade Infected)* Day 19** Bacterial Blight	Day 19** Leaf Blotch	Day 32** Bacterial Blight	Day 32** Leaf Blotch
		Flag Leaf		
Fortuna	.00b	.01a	.13b	.17ab
Thatcher	.00b	.00a	.78a	.41a
Crim	.01ab	.01a	.19b	.15abc
Polk	.00b	.00a	.12b	.01d
Neepawa	.00b	.00a	.08b	.04bcd
Waldron	.00b	.00a	.16b	.10bcd
Justin	.00b	.00a	.41b	.00e
Selkirk	.00b	.00a	.13b	.11bcd
Manitou	.00b	.00a	.42b	.01d
Chris	.00b	.00a	.43b	.01d
Sheridan	.00b	.00a	.39b	.00e
Era	.00b	.00a	.07b	.00e
Hercules	.00b	.00a	.43b	.00e
RR-68	.00b	.00a	.34b	.03bcd
Leeds	.06a	.00a	.00b	.00e
		Second Leaf		
Fortuna	7.1a	2.1a	3.7bcd	4.4a
Thatcher	.00d	.00b	14.8a	3.4ab
Crim	.39bc	.01b	3.7bcd	2.7abc
Polk	.00d	.00b	1.9cde	.91d
Neepawa	.00d	.00b	6.1b	3.1ab
Waldron	.01d	.00b	3.6bcd	2.3bc
Justin	.00d	.00b	1.4cde	.91cd
Selkirk	.09bcd	.01b	4.0bc	2.4bc
Manitou	.02cd	.00b	.92e	.70d
Chris	.06cd	.00b	1.2de	.52de
Sheridan	.01d	.00b	2.4cde	3.1ab
Era	.00d	.00b	.80e	.47de
Hercules	.00d	.00b	.04f	.15de
RR-68	.62b	.00b	.93e	.23de
Leeds	.00d	.00b	.03f	.03e

Table 7 - continued.

Cultivars	Disease Rating (% Lead Blade Infected)* Day 19** Bacterial Blight	Day 19** Leaf Blotch	Day 32** Bacterial Blight	Day 32** Leaf Blotch
	Third Leaf			
Fortuna	1.9ab	.89a	26.7a	3.7ab
Thatcher	.15bc	.03c	25.8a	3.5ab
Crim	2.0a	.31bc	25.7a	3.1abc
Polk	.14bc	.09bc	25.1a	2.3bcd
Neepawa	.54abc	.45ab	21.4ab	2.9abc
Waldron	.05c	.03c	21.1ab	3.3abc
Justin	.25bc	.03c	16.2abc	2.9abc
Selkirk	1.1ab	.29bc	15.1abc	3.1abc
Manitou	.33bc	.20bc	15.0abc	2.0cd
Chris	.61abc	.17bc	8.7bcd	1.5de
Sheridan	.10bc	.24bc	8.6bcd	4.4a
Era	.24bc	.19bc	5.7cd	2.4bcd
Hercules	.00d	.00c	3.2de	1.2e
RR-68	.84abc	.07c	3.0de	.97e
Leeds	.00d	.00c	.10e	.31f

* Readings for both diseases taken on same leaf. Means of 30 leaves covered by same letter are not significantly different at the .05 level using Duncan's Multiple Range test.

** Number of sampling days after first inoculation.

Those cultivars with the lowest rates of increase for both diseases were Leeds, Hercules, RR-68, and Chris (Table 8). Those cultivars with the highest rates of increase for bacterial blight were Thatcher, Waldron, Polk, Selkirk, and Justin; while the highest rates of increase for leaf blotch occurred on Fortuna, Thatcher, Selkirk, Polk, and Justin. The rate of increase of bacterial blight was generally higher than the corresponding leaf blotch increase on the

same leaves. Rate of increase of Xanthomonas is higher as one proceeds from the flag leaf to the 3rd leaf on the same plant.

Table 8. Effect of cultivar resistance on rate of increase on leaf blotch and bacterial blight.

	Rate of Increase (r)*							
	Leaf Blotch				Bacterial Blight			
Cultivar	Flag Leaf	Second Leaf	Third Leaf	Mean	Flag Leaf	Second Leaf	Third Leaf	Mean
Thatcher	.18	.28	.12	.19	.21	.36	.41	.33
Polk	.00	.21	.26	.16	.11	.25	.41	.26
Waldron	.00	.15	.14	.10	.14	.30	.39	.28
Selkirk	.11	.26	.21	.19	.11	.29	.37	.26
Justin	.00	.21	.24	.15	.18	.24	.37	.26
Era	.00	.19	.09	.09	.10	.21	.31	.21
Fortuna	.13	.29	.19	.20	.11	.28	.30	.23
Crim	.13	.27	.11	.17	.14	.23	.30	.22
Sheridan	.00	.28	.21	.16	.18	.20	.28	.22
Neepawa	.07	.28	.21	.19	.10	.31	.25	.22
Manitou	.00	.20	.19	.13	.18	.21	.22	.20
Hercules	.00	.14	.23	.12	.18	.00	.22	.13
Chris	.00	.19	.21	.13	.18	.23	.19	.20
RR-68	.00	.14	.22	.12	.16	.21	.17	.18
Leeds	.00	.05	.16	.07	.00	.02	.02	.01

* $$r = \frac{\log_e \frac{x_2}{1-x_2} - \log_e \frac{x_1}{1-x_1}}{t_2 - t_1}$$ where x_1 = disease rating at date 1 (t_1) and x_2 = disease rating at date 2 (t_2)

To clarify the relationships between leaf blotch and bacterial blight, regression, and correlation coefficients were calculated (Table 9). No coefficients were calculated on sampling date 19 for the flag and 2nd leaves because only trace percentages of infection were present on most cultivars. On day 32 the flag leaf showed no significant correlation between leaf blotch and bacterial blight. The

2nd leaf, 3rd leaf, and the whole plant data for both dates showed highly significant correlation between the two diseases. Also for all treatments and dates of sampling it was shown that for every unit of leaf blotch there corresponded 1.74-5.83 units of bacterial blight infection. This is in agreement with observations from inoculation and cultivar screening experiments; that is, the higher the leaf blotch infection the higher the corresponding bacterial blight infection on the same plant.

Table 9. Relationships between leaf blotch and bacterial blight as indicated by correlation (r) and regression (b).

Readings On		Date of Sampling (Days after inoculation) Day 19	Day 32
Individual Leaf Data	Flag Leaf	---	r = 0.44 ; b = 1.74
	Second Leaf	---	r = 0.67**; b = 1.77
	Third Leaf	r = 0.73**; b = 2.36	r = 0.67**; b = 5.83
Whole Plant Data	Flag + Second + Third Leaves	r = 0.95**; b = 3.76	r = 0.77**; b = 4.10

** Significant values of r are at the .01 level.
Values of b are read as units of Xanthomonas infection/unit of Septoria infection based on % leaf area infected by both diseases.

Chemical Control- The relative effectiveness of pesticides (Table 10) was assessed in the laboratory by a spore germination bioassay using S. avenae f. sp. triticea. A glass microscope slide was coated with a thin layer of collodion (Mallinckrodt's 2 1/2% in amyl acetate) as follows: one drop of collodion was deposited on the

surface of water in a dish and allowed to disperse; the slide was then dipped into the film and allowed to air dry.

Table 10. Pesticide formulations used in laboratory and field studies.

Trade Name	Chemical Name	Active Ingredient	Field Dosage (Kg/hectare)
Agri-mycin 17 (Pfizer)	Streptomycin (from Streptomycin sulfate)	17%	0.322
Benlate (Dupont)	Benomyl [Methyl 1-Butylcabamoyl] -2- benzimidazolecarbamate	50%	2.241
Difolitan (Chevron)	Cis-N- [(1, 1, 2, 2-tetrachloroethyl) thio] - 4 - cyclohexene-1, 2- dicarboximide	80%	2.241
Dyrene (ChemAgro)	2,4-Dichloro-6-(o-chloroanilino) -s-triazine	50%	2.241
Manzate 200 (Dupont)	Zinc (2%) and Maganese (16%) ethylene bisdithiocarbamate - coordination product	80%	2.241
Polyram (Niagara)	A mixture of 5.2 parts by weight (83.9%) of ammoniates of [ethylenebis (dithicarbamato)] -zinc with 1 part by weight (16%) ethylenebis- [dithio-carbamic acid] bimolecular and trimolecular cyclic anhydrosulfides and disulfides	80%	2.241

One drop of spore suspension ($4\text{-}5 \times 10^5$ spores/ml) and one drop of a 2x concentration of the test chemical were mixed together on a collodion coated microscope slide. The several concentrations of each of the 6 chemicals were replicated 6 times. Microscopic observations were made after 24 hours and spores with germ tubes longer than the

width of the spores were recorded as germinated. Dosage-response curves were constructed by plotting the percentage of spores not germinated (Table 11) against chemical concentration of each chemical on logarithmic-probability graph paper (2 log cycles) (Figures 3, 4).

Table 11. Inhibition of Septoria avenae f. sp. triticea spore germination by pesticides.

	Concentration µg/ml							
Treatment	250	125	100	50	25	12.5	5.0	ED_{50} Values (µg/ml)
			Control*					
Streptomycin	74.4a	36.4b	0c					162
Benlate		70.0a	6.5b	0b				120
Polyram					98.0a	18.5b	0c	15.5
Manzate					77.4a	35.2b	0c	15.8
Dyrene	100	100	100	100	100	100	100	---
Difolitan	100	100	100	100	100	100	100	---

* Percent not germinated

Dyrene and Difolitan were the most toxic fungicides to S. avenae f. sp. triticea. These two compounds completely inhibited spore germination at all dosages tested. Manzate 200 was equal to Polyram in its fungitoxic properties, but both materials were inferior to Dyrene and Difolitan. Benlate was more effective than Streptomycin. Effective dose 50 values (ED_{50}), determined by graphic methods, are the most appropriate criteria for comparing the pesticides.

The six pesticides were bioassayed against X. translucens by measuring inhibition zones on seeded King's Medium B agar after 24 hours growth. Plates were seeded by atomizing a water suspension of

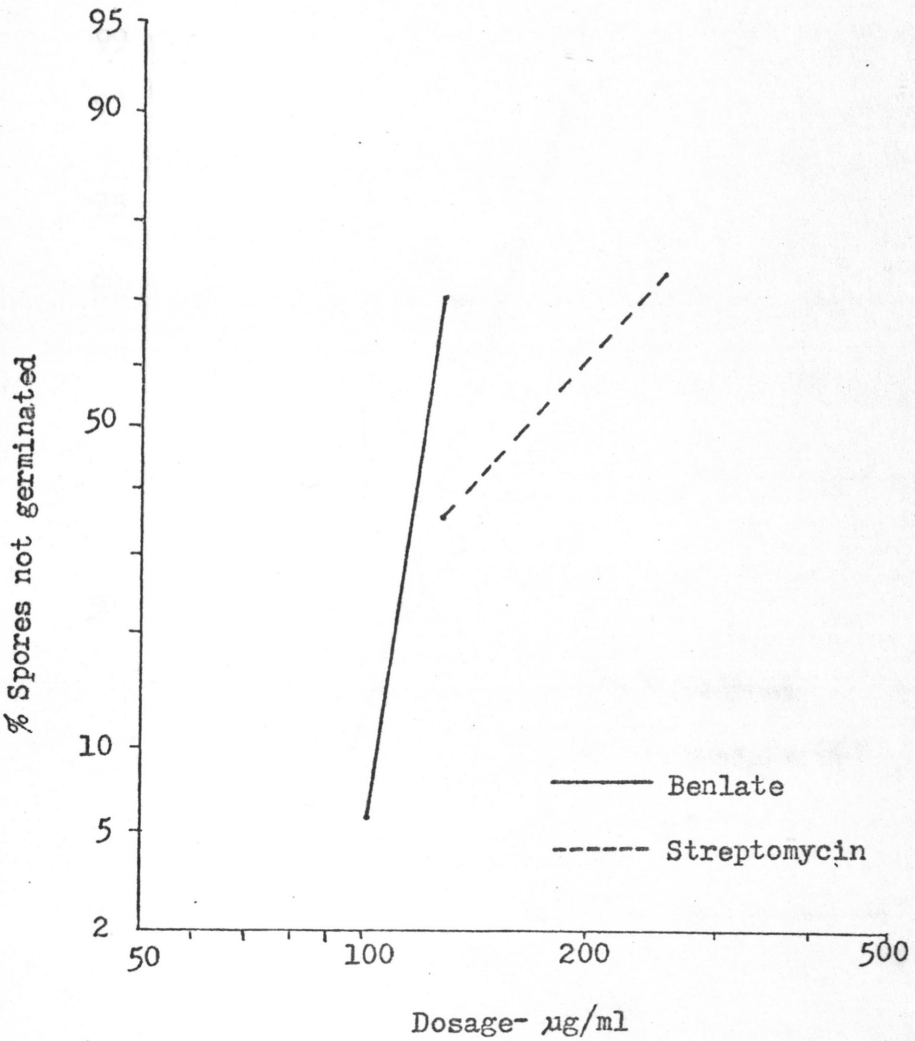


Figure 3. Dosage response curves for Benlate and Streptomycin against Septoria avenae f. sp. triticea spore germination.

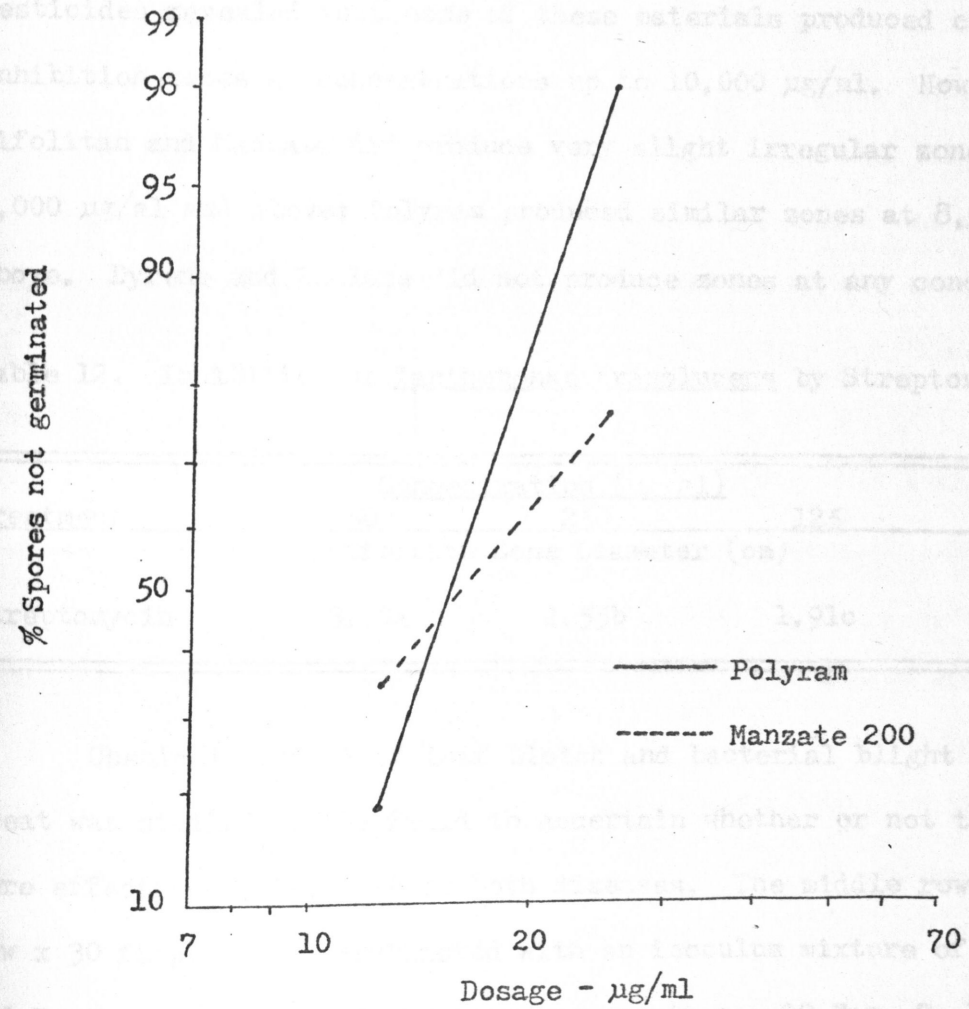


Figure 4. Dosage response curves for Polyram and Manzate 200 against Septoria avenae f. sp. triticea spore germination.

bacteria onto the agar surface. Autoclaved #3 cork borer filter paper discs, 7mm in diameter, were soaked with 36 µl of the desired concentrations of each chemical and then placed on the seeded plates.

Streptomycin impregnated discs produced clear-cut inhibition zones at all concentrations equal to or greater than 62.5 µg/ml, the lowest concentration tested (Table 12). Further tests with the other pesticides revealed that none of these materials produced clear-cut inhibition zones at concentrations up to 10,000 µg/ml. However, Difolitan and Manzate did produce very slight irregular zones at 6,000 µg/ml and above; Polyram produced similar zones at 8,000 µg/ml and above. Dyrene and Benlate did not produce zones at any concentration.

Table 12. Inhibition of Xanthomonas translucens by Streptomycin.

Treatment	Concentration (µg/ml) 500	250	125	62.5
	Inhibition Zone Diameter (cm)			
Streptomycin	3.10a	2.55b	1.91c	1.22d

Chemical control of leaf blotch and bacterial blight on Chris wheat was studied in the field to ascertain whether or not the chemicals were effective against one or both diseases. The middle row of each 7 row x 30 ft plot was air-blasted with an inoculum mixture of Septoria and Xanthomonas on the following four evenings: 29 May, 9, 17, and 29 June. These rows provided disease foci from which natural spread could occur. Pesticides were applied with a 30 gallon John Bean "Spartan" sprayer with an 8 ft boom calibrated to deliver 934.9 liters/ha at

100 psi on the mornings of 11, 22, June and 2 July. The experiment was conducted in a randomized complete block design replicated 5 times with 8 treatments. Plots were sampled on the mornings of 9, 17, 29, June and 10 July by removing ten-culms from each plot. These were taken to the laboratory and individual leaves were rated visually for disease symptoms.

Benlate and Polyram controlled leaf blotch on 3rd leaves 7 days after the first application (Table 13). On day 19, Benlate, Manzate, Streptomycin, and Dyrene controlled leaf blotch on the 3rd leaves. Polyram was not significantly effective at this time. On second leaves only Dyrene provided significant control, although all treatments except Polyram and Difolitan tended to be effective. Similar trends were observed on flag leaves.

Bacterial blight readings were so variable that evaluation of the data was difficult. Only Dyrene controlled bacterial blight significantly and only on the 3rd leaf 19 days after the first spraying. Streptomycin, Manzata and Benlate tended to be effective at this time.

Again the Xanthomonas infection was higher than Septoria infection on the same leaf (based on means per respective leaves) and relatively high levels of leaf blotch were generally accompanied by high levels of bacterial blight.

The effect of chemical treatments on disease increase in the field was evaluated by calculating r values (33). Both diseases increased more rapidly on upper leaves and bacterial blight generally increased more rapidly than leaf blotch on all leaves (Table 14).

Table 13. Influence of pesticides upon leaf blotch and bacteria blight severity on Chris wheat under field conditions.

Treatment	Disease Rating (% Leaf Blade Infected)* Day 7** Bacterial Blight	Day 7** Leaf Blotch	Day 19** Bacterial Blight	Day 19** Leaf Blotch
	Flag Leaf			
Difolitan	.01a	.00	3.2a	.48a
Polyram	.01a	.00	2.2ab	.23ab
Control (not sprayed)	.01a	.00	2.2ab	.26ab
Manzate	.01a	.00	1.8b	.20ab
Benlate	.00a	.00	1.6b	.19ab
Manzate + Benlate + Streptomycin	.03a	.00	2.1ab	.20ab
Streptomycin	.01a	.00	1.5b	.15b
Dyrene	.01a	.00	1.9b	.03b
Means	.01	.00	2.1	.22
	Second Leaf			
Difolitan	.06a	.02ab	8.7a	1.6a
Polyram	.08a	.01b	3.9b	.92b
Control (not sprayed)	.22a	.07ab	5.1ab	.91b
Manzate	.30a	.03ab	2.8b	.66bc
Benlate	.27a	.17a	3.3b	.55bc
Manzate + Benlate + Streptomycin	.50a	.09ab	4.5b	.75bc
Streptomycin	.15a	.01b	3.3b	.51bc
Dyrene	.07a	.01b	2.8b	.35c
Means	.21	.05	4.3	.78
	Third Leaf			
Difolitan	6.0a	1.2a	42.7a	3.8ab
Polyram	3.1a	.54b	39.9ab	3.1ab
Control (not sprayed)	5.0a	1.2a	37.5abc	3.9a
Manzate	3.3a	.86ab	25.3abcd	2.0bc
Benlate	2.3a	.54b	24.9abcd	2.0bc
Menzate + Benlate + Streptomycin	5.8a	.95ab	22.4bcd	1.7c
Streptomycin	4.0a	1.2a	20.3cd	2.0bc
Dyrene	3.9a	1.1ab	19.0d	2.0bc
Means	4.2	.95	29.0	2.6

* Means of 50 leaves covered by same letter are not significantly different at the .05 level using Duncan's Multiple Range test.

** Days after first spraying.

Table 14. Effect of pesticides on rate of increase of leaf blotch and bacterial blight on Chris wheat.

Leaf Treatment	Rate of Increase (r)*					
	Flag Leaf		Second Leaf		Third Leaf	
	Leaf Blotch	Bacterial Blight	Leaf Blotch	Bacterial Blight	Leaf Blotch	Bacterial Blight
Difolitan	.32	.48	.36	.42	.19	.18
Polyram	.25	.45	.37	.33	.18	.27
Control	.28	.45	.21	.27	.19	.27
Manzate	.25	.43	.26	.19	.16	.25
Benlate	.25	.42	.09	.20	.16	.25
Manzate + Benlate + Streptomycin	.25	.35	.18	.19	.16	.25
Streptomycin	.25	.42	.32	.23	.10	.24
Dyrene	.09	.44	.31	.31	.11	.24

* $r = 1/t_2-t_1 \left(\log e \frac{x_2}{1-x_2} - \log e \frac{x_1}{1-x_1}\right)$ where x_1 = disease rating at date 1 (t_1) and x_2 = disease rating at date 2 (t_2)

DISCUSSION

Atypical lesions of wheat consistently yielded a bacterium identified as Xanthomonas translucens and a fungus tentatively identified as Septoria avenae f. sp. triticea. Sporulation of the fungus was induced by growing it on V-8 agar under, continuous 100 ft-c or more, fluorescent light. Repeated single spore transfers produced isolates that sporulated in 13 days. A. L. Hooker, and Scharen and Krupinsky isolated and cultured S. avenae and S. nodorum in a similar manner (17, 29).

Pycnidial size (133-160 μ dia) was at the upper limit of that described by Hosford et al. (18) and by Johnson (20). Pycnidiospore length (14.6-20.0 μ) was at the lower limit reported by both sources above. Width and septation of pycnidiospores were similar to descriptions by both sources.

Greenhouse inoculation tests were conducted to determine if inoculation with a mixture of X. translucens and S. avenae f. sp. triticea would result in symptoms similar to those observed under field conditions in 1969. In these tests typical field symptoms were never reproduced. Differences between artificially and naturally occurring symptoms might be explained on the basis of pre-inoculation environment. In this regard Schnathorst concluded that environment prior to inoculation affected plant reaction to various species of Xanthomonads. Subdued light and moderate temperatures resulted in typical lesion development from X. phaseoli infection on bean (30).

In greenhouse experiments the presence of Septoria spores in the inoculum increased Xanthomonas infection. Hyphae of Septoria penetrating through the leaf stomata may carry the bacterium into the host tissue, thereby providing a more effective avenue of entry. This supports the conclusion of Boosalis (2) who indicated that other fungus diseases increased the severity of bacterial blight. Scharen came to similar conclusions in his study on population trends of X. phaseoli in susceptible and resistant hosts (26).

Although Xanthomonas infection was greatest when a mixture of inoculum was used, Septoria infection was greatest when Septoria inoculum was used alone.

Under field conditions Septoria and Xanthomonas infections were generally higher when a mixture of inoculum was used. Few interrelationships between bacterial blight and leaf blotch were observed in the field. If Xanthomonas is carried into plants by penetrating hyphae of S. avenae f. sp. triticea, it might be expected that rusts and other fungi would be equally effective. The abundance of these pathogens could obscure any effect of the relatively infrequent penetrations of S. avenae f. sp. triticea. In most experiments X. translucens infection was higher than the Septoria infection on the same leaf and the higher the severity of leaf blotch the higher the severity of bacterial blight.

In one case, the greenhouse study of cultivar resistance, leaf blotch readings were higher than those for bacterial blight. Evidently, environmental conditions in the greenhouse tests were better suited for Septoria development than Xanthomonas development.

Field experiments complimented greenhouse work in that those cultivars resistant to one disease were also resistant to the other, and conversely, those susceptible to one were susceptible to the other disease. Those cultivars most resistant to both diseases were Leeds, Hercules, Era, and Chris; while those most susceptible to both diseases were Thatcher, Fortuna, Crim, Waldron and Neepawa. The data, along with correlation and regression coefficients, indicate a positive relationship between the pathogencity of the two organisms in the cultivars tested.

It was unfortunate that the cultivar Chris was chosen as the test wheat used in the chemical control and inoculation experiments as it displayed fair resistance to both diseases. Chris was used because of its resistance to stem and leaf rusts.

In the laboratory paper disc bioassay, against X. translucens, Streptomycin was the only effective chemical. All other pesticides (Benlate, Polyram, Manzate, Dyrene, and Difolitan) were tested at 5,000 to 10,000 µg/ml. Dyrene and Benlate showed no zones of inhibition while Polyram showed very slight irregular zones down to 8,000 µg/ml as did Difolitan and Manzate down to 6,000 µg/ml.

In the spore germination bioassay, Dyrene and Difolitan were the most effective pesticides against S. avenae f. sp. triticea. Manzate and Polyram were intermediate while Benlate and Streptomycin were the least effective.

The spore germination bioassay was not consistent with field results. The spore germination bioassay indicated that Benlate and Streptomycin were the least effective treatments; however, they were

fairly effective against leaf blotch in the field. Difolitan and Polyram were effective against S. avenae f. sp. triticea spore germination; whereas, they were consistently ineffective against leaf blotch in the field.

Laboratory bioassays are a more precise means of evaluating the effectiveness of pesticides against plant pathogens because the environment can be closely controlled. Contrasting results between laboratory bioassays and field control experiments are not uncommon. Field performance, however, is the ultimate test of a pesticide and is influenced by several external factors such as weathering and the constant erosion of effective particles due to sorption by other leaf contaminants.

In the field chemical control experiment, Dyrene and Manzate tended to be effective against bacterial blight; however, in the paper disc bioassay they were not effective against bacterial blight. These pesticides were effective against S. avenae f. sp. triticea in both laboratory and field experiments. These results give support to the conclusion that X. translucens is carried into host tissue by penetrating hyphae of S. avenae f. sp. triticea. Since the leaf blotch infection was controlled, the severity of bacterial blight infection was also reduced.

It was demonstrated that chemicals that controlled one disease gave fair control of the other disease, and conversely, that chemicals that had little effect on one disease had very little effect on the other disease. Dyrene and Manzate provided the best control of both diseases while Difolitan and Polyram were not effective against either disease.

CONCLUSIONS

1. Septoria avenae f. sp. triticea cultures on V-8 agar produced pycnidia after exposure to 100 ft-c fluorescent light.
2. Artificial inoculation with Xanthomonas translucens and Septoria avenae f. sp. triticea resulted in mixture of symptoms typical of each disease.
3. The hypothesis that a synergistic relationship exists between the two diseases was supported by the following:
   a) In most experiments Xanthomonas infection resulting from the inoculum mixture of Xanthomonas and Septoria was greater than the amount of infection resulting from inoculum containing only Xanthomonas.
   b) Severity of Xanthomonas infection was higher than Septoria infection on the same leaf and the higher the Septoria infection, the higher the Xanthomonas infection that occurred.
   c) Cultivars resistant to one disease were resistant to the other disease, and conversely, those cultivars susceptible to one disease were also susceptible to the other disease.
   d) Pesticides that were effective against one disease were also effective against the other disease, and conversely, those pesticides not effective against one disease were not effective against the other disease.

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